PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 June 2000 (23.06.00)	
International application No. PCT/AU99/00989	**Applicant's or agent's file reference** 2231646--TDO
International filing date (day/month/year) 09 November 1999 (09.11.99)	**Priority date** (day/month/year) 09 November 1998 (09.11.98)
Applicant KENT, Stephen et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 June 2000 (08.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

09/831307

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

SLATTERY, John, M.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year)
03 September 2001 (03.09.01)

Applicant's or agent's file reference
2231646--TDO

IMPORTANT NOTIFICATION

International application No.
PCT/AU99/00989

International filing date (day/month/year)
09 November 1999 (09.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
VIRAX HOLDINGS LIMITED
THE AUSTRALIAN NATIONAL UNIVERSITY

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
VIRAX IMMUNOTHERAPEUTICS PTY LTD
Suite 220, Kew Junction Tower
89 High Street
Kew, VIC 3101
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[X] other: The Australian National University

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
Cécile CHATEL (Fax 338.87.40)
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

SLATTERY, John, M.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
21 December 2000 (21.12.00)

Applicant's or agent's file reference
2231646--TDO

IMPORTANT NOTIFICATION

International application No.
PCT/AU99/00989

International filing date (day/month/year)
09 November 1999 (09.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED
COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
VIRAX HOLDINGS LIMITED
Kew Junction Tower
Suite 220
89 High Street
Kew, VIC 3101
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
C. Cupello
Telephone No.: (41-22) 338.83.38

PCT/AU99/00989

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

SLATTERY, John, M.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

FRIDAY, - 5 JAN 2001

Date of mailing (day/month/year) 21 December 2000 (21.12.00)	
Applicant's or agent's file reference 2231646--TDO	**IMPORTANT NOTIFICATION**
International application No. PCT/AU99/00989	**International filing date** (day/month/year) 09 November 1999 (09.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION	AU	AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
VIRAX HOLDINGS LIMITED Kew Junction Tower Suite 220 89 High Street Kew, VIC 3101 Australia	AU	AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer C. Cupello
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

003738194

WO 00/28003
PCT/AU99/00989

PCT[illegible]ENT COOPERATION TREA[illegible]

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
SLATTERY, John, M.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

TUESDAY, 30 MAY 2000

Date of mailing (day/month/year) 18 May 2000 (18.05.00)	
Applicant's or agent's file reference 2231646--TDO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/AU99/00989	09 November 1999 (09.11.99)	09 November 1998 (09.11.98)

Applicant
THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,MA,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   18 May 2000 (18.05.00) under No. WO 00/28003

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

THURSDAY 15 JUN 2000

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: Agent :

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

PCT

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	14 JUN 2000 (14/6/00)
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
2231646	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU99/00989	9 NOV 1999 (9/11/99)	9 NOV 1998 (9/11/98)

Applicant

MacFarlane Burnet Centre for Medical Research Limited; The (et al

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

8 JUN 2000 (8/6/00)

2. That date of receipt is:

☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

☐ the date on which this Authority has, in response to the Invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **Attention:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the elections(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide, Volume II.*

☐ *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU **AUSTRALIAN PATENT OFFICE** **PO BOX 200, WODEN ACT 2606, AUSTRALIA** E-mail: pct@ipaustralia.gov.au Facsimile No. 02 6285 3929	Authorized officer JOHN COLDWELL Telephone No. 02 6283 2357

Form PCT/IPEA/402 (July 1998)

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

EA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 2231646/TDO

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/AU99/00989	(09.11.1999) 9 November, 1999	(09.11.1998) 9 November, 1998

Title of invention: Avipox vector coding an HIV antigen and a cytokine

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*
THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED
Yarra Bend Road
Fairfield 3078
Victoria
Australia

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*
COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
Limestone Avenue
Campbell 2612
Australian Capital Territory
Australia

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*
THE AUSTRALIAN NATIONAL UNIVERSITY
Acton 0200
Australian Capital Territory
Australia

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

[X] Further applicants are indicated on a continuation sheet.

See Notes to the demand form

International application No.
PCT/AU99/00989

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

KENT, Stephen
9 St John's Avenue
Camberwell 3124
Victoria
Australia

State *(that is, country)* of nationality: AUSTRALIA

State *(that is, country)* of residence: AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

BOYLE, David Bernard
6 Mary Place
Leopold 3224
Victoria
Australia

State *(that is, country)* of nationality: AUSTRALIA

State *(that is, country)* of residence: AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

RAMSHAW, Ian Allister
28 Kallara Close
Duffy 2611
Australian Capital Territory
Australia

State *(that is, country)* of nationality: AUSTRALIA

State *(that is, country)* of residence: AUSTRALIA

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

Sheet No. 3

International application No.
PCT/AU99/00989

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SLATTERY, John M
HUGHES, E John L
CAINE, Michael J

DAVIES COLLISON CAVE
1 Little Collins Street
Melbourne 3000
Victoria
Australia

Telephone No.: +61 3 9254 2777

Facsimile No.: +61 3 9254 2770

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[X] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 4

International application No.
PCT/AU99/00989

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

.....
SLATTERY, John M
on behalf of the applicants

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *day/month/year*	25 OCT 2000
Applicant's or agent's file reference 2231646 TDO	**IMPORTANT NOTIFICATION**

International application No.	International filing date	Priority date
PCT/AU99/00989	9 November 1999	9 November 1998

Applicant
THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	CRAIG ALLATT Telephone No. (02) 6283 2414

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2231646	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU99/00989**	International filing date *(day/month/year)* 9 November 1999	Priority Date *(day/month/year)* 9 November 1998

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C12N 7/00 A61K 39/275

Applicant

THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
8 June 2000	24 October 2000
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **CRAIG ALLATT** Telephone No. (02) 6283 2414

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
[redacted]/AU99/00989

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1 - 38**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims	**YES**
	Claims **1 - 38**	**NO**
Industrial applicability (IA)	Claims **1 - 38**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 WO 92/22641
D2 WO 94/16716
D3 J Ruby et al
D4 J Kim et al

Inventive Step (IS)

1. Claims 1 - 38 lack an inventive step in light of D1 when read in light of D3. D3 discloses the effect of cytokine expression by a recombinant vaccinia virus on the course of a disease. This document indicates that coexpression of cytokines in recombinant viruses increases the effectiveness of the vaccination. D1 discloses recombinant poxvirus vaccines expressing HIV antigens. A skilled addressee wanting to make a recombinant vaccine against the HIV virus with increased effectiveness, aware of D3, and coming across D1 would recognise that making a recombinant virus expressing with a cytokine and a HIV antigen would lead to a more effective HIV vaccine. In light of both D1 and D3 there is no inventive skill in selecting suitable cytokines or HIV antigens for use in the vaccine, nor in the construction of the final recombinant vaccine. Consequently general claims to avipox viruses encoding cytokines and HIV antigens do not appear to involve an inventive step.
2. Claims 1 - 38 lack an inventive step in light of D2. D2 discloses the use of poxvirus vectors to express a cytokine and a tumour associated antigen. A skilled addressee wanting to make a poxvirus vaccine for HIV and finding this document would recognisee that the same type of effect will be achieved with any antigen. I.e. it is possible to substitute one antigen for another, regardless of their origin. The skilled addressee would therefore recognise that a tumour associated antigen could be replaced by an HIV antigen to obtain similar benefits,. The substitution of one known antigen for another known antigen does not constitute an inventive step; this merely consists of substituting one "mechanical equivalent" for another.
3. Claims 1 - 38 lack an inventive step in light of D3. D3 discloses the coadministration of two vectors, one encoding a cytokine and the other encoding an HIV antigen. The person skilled in the art wanting to make an improved HIV vaccine and encountering this document would recognise the advantage of coadministering a cytokine expression vector with the HIV vaccine. One obvious change to the system of coadministering two separate vectors is to administer on vector expressing both proteins. There is no technical difficulty in expressing two proteins in the one vector where before both proteins were expressed in separate vectors. Consequently, the combining of two expression systems that were known to be coadministered into one expression system lack an inventive step.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1. The claims are not clear as claims 1 and 9 are of identical scope. Claim 9 is directed to a recombinant viral composition of one component and is therefore considered to be directed to the recombinant virus *per se*. Claim 1 is directed to the same recombinant virus. Consequently, claim 9 is redundant on claim 1, rendering the claims unclear.

PCT REQUEST

2231646–TDO

Original (for **SUBMISSION**) - printed on 09.11.1999 04:41:32 PM

0 **0-1**	**For receiving Office use only** International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	

0-4 **0-4-1**	**Form - PCT/RO/101 PCT Request** Prepared using	**PCT-EASY Version 2.84 (updated 01.07.1999)**
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	**Australian Patent Office (RO/AU)**
0-7	**Applicant's or agent's file reference**	**2231646--TDO**
I	**Title of invention**	**RECOMBINANT VIRAL CONSTRUCTS AND METHODS RELATING THERETO**
II II-1 II-2 II-4 II-5 II-6 II-7 II-8 II-9 II-10	**Applicant** This person is: Applicant for Name Address: State of nationality State of residence Telephone No. Facsimile No. e-mail	 **applicant only** **all designated States except US** **THE MACFARLANE BURNET CENTRE FOR MEDICAL RESEARCH LIMITED** **-** **Yarra Bend Road** **Fairfield, Victoria 3078** **Australia** **AU** **AU** **-** **-** **-**

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III-1	**Applicant and/or inventor**	
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III-5	**Applicant and/or inventor**	
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IV-2-1	Name(s)	HUGHES, E, John, L; CAINE, Michael, J
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT

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V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	**Exclusion(s) from precautionary designations**	NONE
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	09 November 1998 (09.11.1998)
VI-1-2	Number	PP7007
VI-1-3	Country	AU
VI-2	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1
VII-1	**International Searching Authority Chosen**	Australian Patent Office (ISA/AU)

VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	5	-
VIII-2	Description (excluding sequence listing part)	30	-
VIII-3	Claims	5	-
VIII-4	Abstract	1	2231646.txt
VIII-5	Drawings	6	-
VIII-6	Sequence listing part of description	1	-
VIII-7	TOTAL	48	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-15	Nucleotide and/or amino acid sequence listing in computer readable form		separate diskette
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	-	

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VIII-19	**Language of filing of the international application**	English
IX-1	**Signature of applicant or agent**	
IX-1-1	Name (LAST, First)	SLATTERY, John, M

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/AU
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

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(54) Title: AVIPOX VECTOR CODING AN HIV ANTIGEN AND A CYTOKINE

(57) Abstract

The invention relates to a fowl pox virus encoding an HIV antigen (gag and/or pol) and a cytokine (γ–interferon).

A VIPOX VECTOR CODING AN HIV ANTIGEN AND A CYTOKINE

FIELD OF THE INVENTION

The present invention relates generally to recombinant viral constructs expressing a protective antigen together with a cytokine and to vaccine compositions comprising same. In particular, the present invention is directed to a recombinant viral construct capable of inducing an immune response to an HIV antigen and, most particularly an HIV-1 antigen. The present invention is useful, *inter alia*, in the therapeutic and/or prophylactic treatment of HIV.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

There is currently no effective method of treating HIV infection. Current treatment strategies can suppress plasma HIV-1 RNA levels to very low levels, however latently infected cells harbouring HIV-1 DNA remain detectable and viral resistance and relapse is common [1,2]. Treatment-induced reductions in HIV-1 levels results in a loss of antigenic stimulus for effective immune responses. HIV-specific cytotoxic T lymphocyte (CTL) responses, thought to be a critical effector mechanism in the control of HIV-1, decline to low levels following effective anti-HIV therapy [3].

Previous trials of therapeutic HIV-1 vaccines have shown that it is possible to stimulate anti-HIV immune responses in HIV-1 infected individuals, but no clinical benefit has been demonstrated [4-6]. Prior studies have used protein-based HIV-1 vaccines incapable of inducing CTL responses or vaccinated individuals with substantial levels of replicating HIV-1. Even moderate levels of replicating HIV-1 results in a loss of HIV-specific $CD4^+$ T-helper (Th) responses which are required to initiate and sustain an effective CTL

response [7].

Additionally, no preventative HIV vaccines currently exist. Although simple recombinant avipox vaccines (without co-expression of cytokines) can induce CTL responses in a proportion of human and non-human primate subjects, the response is often weak, transient, or non-existant. There is a need for more reliable vaccine vectors for the induction of HIV specific CTL and Th responses.

In work leading up to the present invention, the inventors have determined that the magnitude and phenotype of the specific immune response to HIV can be enhanced by vaccination with a recombinant fowl pox virus construct comprising both an HIV gag/pol encoding nucleic acid molecule and a cytokine encoding nucleic acid molecule, in particular, IFN-γ.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The subject specification contains nucleotide sequence information prepared using the programme PatentIn Version 2.0, presented herein after the bibliography. Each nucleotide sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, etc) and source organism for each nucleotide sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide sequences referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (e.g. <400>1, <400>2, etc).

One aspect of the present invention provides a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV antigen.

Another aspect of the present invention there is provided a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV-1 antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV-1 antigen.

Yet another aspect of the present invention provides a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and the second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Still yet another aspect of the present invention provides a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and a second nucleic acid molecule encoding interferon-γ or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

A further aspect of the present invention provides a recombinant viral construct, comprising a fowl pox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and a

second nucleic acid molecule encoding interferon-γ of functional equivalent thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Another further aspect of the present invention relates to a vaccine comprising a recombinant viral construct which construct comprises a avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV-antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV-antigens.

Still another further aspect of the present invention provides a vaccine comprising a recombinant viral construct which construct comprises an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and a second nucleic acid molecule encoding interferon γ or functional derivative thereof wherein said vaccine is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Still yet another aspect of the present invention provides a pharmaceutical composition for use in inducing, enhancing or otherwise stimulating an immune response to HIV in a mammal comprising a recombinant viral construct as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. The composition may also comprise the recombinant viral construct together with a known antiviral compound or molecule.

Still yet another further aspect of the present invention provides a method of inducing, enhancing or otherwise stimulating an immune response, in a mammal, to HIV said method comprising administering to said mammal an effective amount of a vaccine as hereinbefore defined, for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Still another aspect of the present invention provides a method of inducing, enhancing or otherwise stimulating an immune response, in a mammal, to HIV said method comprising administering to said mammal an effective amount of a recombinant viral construct as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Yet another aspect of the present invention provides a method of treating a mammal, said method comprising administering to said mammal an effective amount of a vaccine as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Yet another aspect of the present invention provides a method of treating a mammal, said method comprising administering to said mammal an effective amount of a recombinant viral construct as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Yet another aspect of the present invention provides a method for the treatment and/or prophylaxis of HIV infection or AIDS in a mammal, said method comprising administering an effective amount of a vaccine as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Yet another aspect of the present invention provides a method for the treatment and/or prophylaxis of HIV infection or AIDS in a mammal, said method comprising administering an effective amount of a recombinant viral construct as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

The present invention further extends to the use of the subject recombinant viral construct in the manufacture of a medicament for the treatment and/or prophylaxis of HIV infection.

Yet another aspect of the present invention provides an agent useful for inducing, enhancing or otherwise stimulating an immune response to HIV said agent comprising a recombinant viral construct as hereinbefore defined.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of the construction of FPVgag/pol-IFNγ.

Figure 2 is a schematic representation of the construction of FPVgag/pol.

Figure 3 is a graphical representation of the safety of FPVgag/pol-IFNγ immunisation. **A.** No significant fever was documented following FPVgag/pol-IFNγ vaccination of macaques. Animals M9 and M10 (◆,■) received FPVgag/pol-IFNγ 10^8 PFU IM, animal M7 (△) received FPVgag/pol, and animals M2, M3, M4 and M5 (○) were unvaccinated controls. **B.** No change in T cell or monocyte counts was observed following FPVgag/pol-IFNγ vaccination of macaques. PBMC obtained from animals vaccinated with FPVgag/pol-IFNγ (M9, M10) or FPVgag/pol (M7) were stained for $CD4^+$ T cells, $CD8^+$ T cells and $CD14^+$ monocytes prior to vaccination (△, 6 times over 8 months prior to vaccination, mean±SD shown) on the day of vaccination (○) and following vaccination (■, weekly for 4 weeks following vaccination, mean±SD shown).

Figure 4 is a graphical representation of enhanced gag-specific Th1 response following FPVgag/pol-IFNγ vaccination of macaques. **A.** T cell proliferative response to p24 antigen was measured serially following 2 vaccinations (arrows) of macaques with FPVgag/pol-IFNγ (animals M9, M10, solid and hatched bars) or FPVgag/pol vaccinations of macaques (animal M7, open bars). **B.** Secretion of IFN-γ and IL-4 by PBMC in response to recombinant HIV-1_{SF2}p24 protein stimulation obtained before and after the first FPV vaccination. FPVgag/pol-IFNγ vaccinated macaques (M9 and M10) and a FPVgag/pol immunised animal (M7) is shown.

Figure 5 is a graphical representation of enhanced Gag/pol specific CTL response following FPVgag/pol-IFNγ vaccination. Quantification of CTL precursors to Env and Gag/pol antigens was analysed following FPV vaccinations. CTL frequencies were assessed following initial and booster FPV vaccinations (arrows). Recognition of control targets expressing vaccinia antigens alone have been subtracted.

Figure 6 is a photographic representation of Gag/pol specific antibodies are enhanced following FPVgag/pol vaccination. Western blotting of serial plasma (1:100 dilution) from animals M7, M9 and M10 following FPV vaccinations (arrows). Strips are labelled with weeks prior to or following the first and second vaccinations. Negative and positive controls represent uninfected and HIV-1 infected humans respectively.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated, in part, on the determination that the immune response to HIV, and in particular HIV-1, can be enhanced when vaccination is performed utilising a recombinant viral construct comprising both a nucleic acid molecule encoding one or more protective HIV antigens, such as gag/pol, and a nucleic acid molecule encoding a cytokine, such as IFN-γ.

Accordingly, one aspect of the present invention provides a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV antigen.

Reference herein to "HIV" should be understood as including reference to any HIV strain including homologues and mutants. In a particularly preferred embodiment said HIV is HIV-1.

According to this preferred aspect of the present invention there is provided a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV-1 antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV-1 antigen.

Reference to "inducing, enhancing or otherwise stimulating" an immune response to an HIV-1 antigen should be understood as stimulating or facilitating the stimulation of a specific immune response. The specific immune response may be a T-cell and/or humoral response which is directed to any one or more peptides or epitopes, respectively, of the HIV antigen encoded by the nucleotide sequence comprising the recombinant viral

construct of the present invention. Preferably, the immune response is a Th-1 and CTL response. However, even where an immune response is skewed to a Th-1 type response, some degree of antibody generation may nevertheless occur.

Reference to "HIV antigen or derivative thereof" should be understood as a reference to any component of HIV or derivative thereof. Said component may be a peptide, polypeptide, protein or non-proteinaceous fragment such as a carbohydrate. It should be understood that the antigen may comprise one or more sites in respect of which a specific immune response is stimulated. For example, processing of the antigen by an antigen presenting cell may result in the production of one or more peptides which are co-expressed with MHC class II and which stimulate specific T helper cells. Similarly, the processing and co-expression of said peptides together with MHC class I may lead to the stimulation of one or more specificities of T cytotoxic cells. Said antigen may also comprise one or more epitopes to which a humoral immune response may be directed. Said epitope may be a linear or a conformational epitope. Where the epitope is a linear epitope, folding of the expressed antigen into its native conformation may not be required to achieve the stimulation of a specific humoral response directed to that epitope. Said antigen may for example comprise only one epitope and may take the form of a hapten. However its co-expression with a cytokine, in accordance with the present invention, may be sufficient to render said hapten immunogenic and therefore suitable for use in the present invention.

Accordingly, it should be understood that reference to stimulating a response to an HIV "antigen" should be understood as a reference to the stimulation of specific immune cells (i.e. T cells and/or B cells) which are directed to one or more sites of the HIV antigen. Examples of antigens suitable for use in the present invention include, but are not limited to, one or more of the molecules encoded by the HIV viral genes *gag*, *pro*, *pol* and *env*. The expression product of each gene is given the same name, but in normal type with the first letter capitalized. Preferably said HIV antigens are the HIV-1 Gag and/or Pol molecules or derivatives thereof.

According to this preferred embodiment there is provided a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and the second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Reference to "cytokine" is a reference to any cytokine or derivative thereof which is capable of modulating the stimulation of an immune response. For example, said cytokine may induce, up-regulate, enhance or maintain an immune response. Particularly preferred cytokines are those which either support a Th1 response, a CTL response or skew a response towards a Th1 type response, for example, IL-2 and γ-interferon or functional equivalents thereof. Preferable said cytokine is γ-interferon.

Accordingly, in a particularly preferred embodiment there is provided a recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and a second nucleic acid molecule encoding interferon-γ or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Avipox viral vectors suitable for use in the present invention may comprise the whole or part of any avipox virus or derivative thereof. The present invention should be understood to include derivatives such as modified virus, for example virus which has been attenuated. Examples of avipox viruses suitable for use in the present invention include, but are not limited to, fowl pox virus and canary pox virus. Preferably said avipox virus is fowl pox virus.

In a most preferred embodiment there is provided a recombinant viral construct, comprising a fowl pox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and a

second nucleic acid molecule encoding interferon-γ of functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Reference to "derivatives" should be understood to include fragments, parts, portions, equivalents, analogs, mutants, homologs, mimetics from natural, synthetic or recombinant sources including fusion proteins. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Additions to amino acid sequences including fusions with other peptides, polypeptides or proteins.

The derivatives include fragments having particular epitopes or parts of the entire protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules. For example, the vector or derivative thereof may be fused to a molecule to facilitate its entry into a cell. Derivatives of nucleic acid sequences may be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid molecules. The derivatives of the nucleic acid molecules of the present invention include oligonucleotides, PCR primers, antisense molecules and fusion of nucleic acid molecules.

Equivalents should be understood to include reference to molecules which can act as a functional analog or agonist. Equivalents may not necessarily be derived from the subject molecule but may share certain conformational similarities. Alternatively, equivalents may be designed to mimic certain immunological and physiochemical properties of the subject molecule. Equivalents may be detected following, for example, natural product

screening. Equivalents also include peptide mimics. Homologs contemplated herein include, but are not limited to, molecules derived from different species. Fragments include portions which are effective in achieving the object of the present invention.

The nucleic acid molecule suitable for use in the present invention may be DNA or RNA. Preferably said nucleic acid molecule is DNA. Reference to the cytokine or HIV antigen encoded by a nucleic acid molecule is a reference to the expression product of said nucleic acid molecule.

Without limiting the present invention to any one theory or mode of action, it is thought that administration of the recombinant construct of the present invention enhances the phenotype and magnitude of the HIV specific T-cell response. It may also result in expansion of the T-cell repertoire directed to the T-cell antigen comprising the construct of the present invention. A protective immune response against HIV-1 (specifically against the HIV-1 antigen comprising the construct) is therefore stimulated in individuals administered the recombinant viral construct of the present invention.

Administration of the subject viral construct in the form of a pharmaceutical composition, may be performed by any convenient means. The viral construct or agent of the pharmaceutical composition are contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 μg to about 1 mg of construct may be administered per kilogram of body weight per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The construct may be administered in any convenient manner such as by the oral, intravenous (where water soluble), intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes or implanting (e.g. using slow release molecules).

Accordingly, another aspect of the present invention relates to a vaccine comprising a recombinant viral construct which construct comprises a avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV-antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV-antigens.

Preferably said HIV-antigens are HIV-1 Gag and/or Pol. Even more preferably said cytokine is interferon-γ.

According to this preferred embodiment there is provided a vaccine comprising a recombinant viral construct which construct comprises an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding HIV-1 Gag and/or Pol or derivatives thereof and a second nucleic acid molecule encoding interferon γ or functional derivative thereof wherein said vaccine is effective in inducing, enhancing or otherwise stimulating an immune response to said Gag and/or Pol.

Most preferably said avipox viral vector is a fowl pox viral vector.

Yet another aspect of the present invention provides a pharmaceutical composition for use in inducing, enhancing or otherwise stimulating an immune response to HIV in a mammal comprising a recombinant viral construct as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. The composition may also comprise the recombinant viral construct together with a known antiviral compound or molecule.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of

microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose,

lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

Still another aspect of the present invention provides a method of inducing, enhancing or otherwise stimulating an immune response, in a mammal, to HIV said method comprising administering to said mammal an effective amount of a vaccine as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Preferably, said HIV is HIV-1.

Still another aspect of the present invention provides a method of inducing, enhancing or otherwise stimulating an immune response, in a mammal, to HIV said method comprising administering to said mammal an effective amount of a recombinant viral construct as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Preferably, said HIV is HIV-1.

Yet another aspect of the present invention provides a method of treating a mammal, said method comprising administering to said mammal an effective amount of a vaccine as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Preferably said HIV antigens are HIV-1 antigens.

Yet another aspect of the present invention provides a method of treating a mammal, said method comprising administering to said mammal an effective amount of a recombinant viral construct as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Preferably said HIV antigens are HIV-1 antigens.

Reference to "mammal" should be understood to include all mammals including primates (e.g. humans, monkeys), livestock animals (e.g. sheep, cows, horses, donkeys, goats, pigs), laboratory test animals (e.g. rats, guinea pigs, rabbits, hamsters), companion animals (e.g. dogs, cats), and captive wild animals (e.g. kangaroos, deer, foxes). Preferably, said animal is a primate and even more preferably a human.

The method of the present invention is useful in the treatment and/or prophylaxis of HIV infection and AIDS. For example, the vaccine of the present invention may be administered into subjects known to be infected with HIV in order induce an immune response against HIV thereby preventing the onset of AIDS. Alternatively, the method of the present invention may be used to reduce serum viral load, to alleviate AIDS symptoms or to induce immunity in mammals thought to be at risk of HIV infection.

The method of the present invention may be particularly useful either early in HIV infection to prevent the establishment of a viral reservoir or for a period after exposure to a possible source of HIV infection.

Yet another aspect of the present invention provides a method for the treatment and/or prophylaxis of HIV infection or AIDS in a mammal, said method comprising administering an effective amount of a vaccine as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Preferably said HIV antigens are HIV-1 antigens.

Still yet another aspect of the present invention provides a method for the treatment and/or prophylaxis of HIV infection or AIDS in a mammal, said method comprising administering an effective amount of a recombinant viral construct as hereinbefore defined for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

Preferably said HIV antigens are HIV-1 antigens.

An "effective amount" means an amount necessary at least partly to attain the desired immune response, or to prevent or to delay the onset or inhibit progression or halt altogether, the onset or progression of a particular condition being treated. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Reference herein to "treatment" and "prophylaxis" is to be considered in its broadest context. The term "treatment" does not necessarily imply that a mammal is treated until total recovery. Similarly, "prophylaxis" does not necessarily mean that the subject will not eventually contract a disease condition. Accordingly, treatment and prophylaxis include amelioration of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylaxis" may be considered as reducing the severity of onset of a particular condition. "Treatment" may also reduce the severity of an existing condition or the frequency of acute attacks.

In accordance with this method, the vaccine of the present invention may be co-administered with a known anti-viral compound or molecule. Such compounds or molecules include, but are not limited to, reverse transcriptase inhibitors (for example, Zidovudine or 3TC) or protease inhibitors (for example, Indinavir). By "co-administered"

is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days between the administration of the vaccine and the known anti-viral compound or molecule. The vaccine and the known anti-viral compound or molecule may be administered in any order.

Routes of administration include but are not limited to intravenously, intraperitoneally, subcutaneously, intracranially, intradermally, intramuscularly, intraocularly, intrathecally, intracerebrally, intranasally, infusion via i.v., drip, and implant. Intramuscular routes are particularly preferred.

The present invention further extends to the use of the subject recombinant viral construct in the manufacture of a medicament for the treatment and/or prophylaxis of HIV infection.

Preferably said HIV infection is HIV-1 infection.

Yet another aspect of the present invention provides an agent useful for inducing, enhancing or otherwise stimulating in a mammal, an immune response to HIV said agent comprising a recombinant viral construct as hereinbefore defined.

Further features of the present invention are more fully described in the following non-limiting Examples. It is to be understood, however, that the detailed description is included solely for the purpose of exemplifying the present invention.

EXAMPLE 1

Animals

Macaques (*M. nemestrina*, aged 24-32 months) were free from SRV infection and anaesthetised with Ketamine (10 mg/kg IM) prior to procedures. The studies were approved by the institutional Animal Experimentation and Ethics Committees.

Seven animals were studied that have been previously described [9,10]. Four animals (M2, M3, M4, M5) served as controls and received no vaccines during the course of this study. These 4 animals (M2-5) had been vaccinated with DNA and FPV HIV-1 vaccines (not containing cytokines) 11-19 months prior to study and resisted a HIV-1 challenge 9 months prior to this study. Three animals (M7, M9, M10) had no previous HIV-1 vaccinations and were infected with HIV-1_{LAI} 9 months prior to this study. Two of these animals (M9, M10) received a novel FPV encoding both gag/pol and human IFNγ and one animal (M7) received a FPV vaccine encoding gag/pol only. Each FPV vaccine was given IM into the anterior thigh at 10^8 PFU in 0.3 ml twice, 3 months apart at 9 and 12 months following HIV-1 infection.

All macaques were previously vaccinated with 3 doses of tetanus toxoid (CSL, Parkville, Australia) IM prior to HIV-1 vaccinations. Each animal was assessed twice daily, following vaccination, for visible swelling or redness at the site of injection and for activity by counting a variety of normal macaque behaviours (individual and conspecific play, foraging, displacement, mounting and grooming activities) as previously described [11]. A 25% reduction in total activity compared to the mean baseline activity in the week prior to vaccination was considered significant. Daily temperature recordings were determined by using an electronic hand held thermometer (Braun Thermoscan) and training the animals to have this applied to their tongues for ≥ 1 second. This method of taking temperatures was found to be 0-0.8°C (mean 0.3°C) lower than rectal temperatures taken on sedated macaques using a rectal thermometer on 22 consecutive occasions.

EXAMPLE 2

Vaccines

The HIV-1 gag/pol genes with or without the human IFNγ gene have been inserted into the FPV genome, along with the *E. coli lacZ* and *gpt* marker genes, between the FPV thymidine kinase (TK) region and the 3' open reading frame (ORF). See Figs 1 and 2.

Plasmid constructs

A. Plasmids for construction of FPV-gag/pol-IFNγ

Construction of these plasmids is shown in Figure 1.

1. The gag/pol genes of HIV-1, isolate ARV-2/SF2, were excised from pUC19.ARV (Chiron Corporation, Emeryville, CA) with SacI and NdeI restriction endonucleases. This fragment (corresponding to nucleotides 684-5132, Genbank Accession No. K02007) was blunt-ended with T4 DNA polymerase and inserted into HincII linearized pBCB07 (12) under the control of the vaccinia P7.5 promoter. This plasmid was names pBC07.ARV.

2. The human IFNγ gene coding sequence was derived by PCR from plasmid pUC9-2 template. pUC9-2 contains the 844 bp Sau3A fragment of human IFNγ cDNA (13). The sense PCR primer was 5'-gcttaattctctc**gggatcgatg** (<400>1). This spans nucleotides 89-111 of the IFNγ cDNA (Genbank Accession No. X13274) with two mismatches introduce to generate a San3A site (bold text). The IFNγ cDNA start codon is underlined. The antisense primer was 5'-attc**gg**atccattac<u>aaaaaag</u>ttgc (<400>2). This spans nucleotides 751-726 of the gene (the antisense strand, downstream of the stop codon at nucleotide 607) with 4 mismatches introduced to generate a BamHI/Sau A site (bold text) and 1 mismatch to generate a TTTTTNT transcription terminator on the sense strand (underlined).

The PCR product was digested with Sau3A and cloned into the BamHI site of pAF09 (14). This places the human IFNγ gene under the control of the FPV P.E/L bidirectional promoter, with the start codon of the gene in frame with the P.E/L start codon. The plasmid was named pDB42a, and also contains the *E. coli lacZ* and *gpt* marker genes, under the

control of the P.E/L and vaccinia P.7.5 promoters, respectively, and the fowlpox TK gene and 3'ORF regions to facilitate recombination.

3. The gag/pol genes and P7.5 promoter were excised from pBC07.ARV by EcoRI digestion, blunted with T4 polymerase, and cloned into SmaI linearized pDB42a. This plasmid, pDB42a.gag/pol, was used for construction of FPV-gag/pol-h IFNγ.

B. Plasmids for construction of FPV-gag/pol

Construction of these plasmids is shown in Figure 2.

1. The gag/pol genes in HIV-1 were derived by PCR with pUC19.ARV (see 1 above) as template. The sense PCR primer was 5'-taatt**atcgat**aataaatgggtgcgagagcg (<400>3). This contains sequence from the P.E/L promoter and nucleotides 791-805 of the HIV-1 gag/pol gene (Genbank Accession No. K02007). The start codon of the gag gene product is underlined the bold text indicates a ClaI site. The antisense primer was 5'-aaa**ggatcc**tttagctttcttaaaaaaaaacatatgg (<400>4). This spans nucleotides 5164-5128 of the gene (on the antisense strand, downstream of the pol stop codon at nucleotide 5101). Two mismatches were introduced to generate a BamHI site (bold text) and 4 mismatches to generate 2 overlapping TTTTTNT transcription terminators on the sense strand (underlined).

The PCR product was digested with ClaI and BamHI and cloned between the ClaI and BamHI sites of pAF04 (D. Boyle, personal communication). This places the gag/pol genes under the control of the FPV P.E/L bidirectional promoter, aligned so that the native start codon of the gag gene is utilised. This is essential for expression and myristilation of the gag gene product. The plasmid was namedpDB43, and also contains the *E. coli gpt* marker gene, under the control of the vaccinia P7.5 promoter, and the FPV TK *t*gene and 3'ORF regions to facilitate recombination.

Virus construction

Recombinant viruses were constructed by standard methods (15), by transfecting FPV-M3 infected chick cells with pDB42a.gag/pol or pDB43. Homologous recombination between both the *t*gene and the 3' ORF regions of the plasmids and the wild type virus results in insertion of HIV gag/pol and *gpt* genes (with and without the h IFNγ and *lacZ* genes) into the virus. The *gpt* gene confers resistance to mycophenolic acid, recombinant viruses were amplified and selected after several rounds of plaque purification in the presence of mycophenolic acid. PCR with primers complementary to sequences flanking the insertion site was used to confirm the absence of wild-type parent virus.

EXAMPLE 3

Blood cell counts and plasma biochemistry

To determine whether the vaccine-expressed IFNγ resulted in abnormalities in plasma biochemistry or blood cell counts, a battery of biochemical and cellular analyses were performed on serial blood samples from the macaques. Multiparameter biochemical analyses and blood counts were performed on automated machines and counts confirmed manually. White cell counts and differential were confirmed by manual counting. PBMC obtained before and after vaccinations were stained for monocyte and T cell markers and analysed by flow cytometry as previously described [9] using antibodies directed against CD2 (Leu5-PE, Becton Dickinson, San Jose, CA), CD4 (OKT4-FITC, Ortho Diagnostics, Raritan, NJ), CD8 (Leu2a-FITC, Becton Dickinson), and CD14.

EXAMPLE 4

HIV-1 Antibody and Th responses

Plasma was assessed for HIV-1 antibodies by particle agglutination (Serodia-HIV, Fujirebio, Japan) and Western blotting using 200μg of standard mixed HIV-1 protein stock [9]. Lymphoproliferative responses were assessed by standard ^{3}-H-thymidine incorporation assay as described [9]. Macaque PBMC in triplicate wells at 10^5 cells/well were stimulated for 6 d with 10μg/ml of recombinant HIV-1_{SF2} gp120 or HIV-1_{SF2}p24

(Chiron) in media containing 10% autologous heat-inactivated serum and pulsed with ^{3}H-thymidine before β-counting. PBMC were also incubated with media alone or media supplemented with 10μg/ml culture derived control antigens to assess unstimulated control responses, and stimulated with PHA (10μg/ml) or tetanus toxoid antigen (0.01 Lf/ml) as positive mitogenic and antigenic control responses. Proliferation is expressed as stimulation index (SI, mean ^{3}H-thymidine incorporation of cells stimulated with antigen/mean incorporation in absence of antigenic stimulation). Supernatants from selected lymphoproliferative cultures were assayed for the presence of IL-4 and IFNγ by EIA (Genzyme, Cambridge, MA).

EXAMPLE 5

Quantitative HIV-specific CTL analyses

Analysis of CTL precursor frequencies to HIV-1 Env and Gag/Pol antigens in macaque PBMC of macaques was performed by a limiting dilution analysis [9]. PBMC were plated in 96 well round-bottomed plates in 7 serial 1.5-fold dilutions of 10^5 to 8.8 – 10^3 cells/well in 24 replicates. Each well was stimulated with 10^4 autologous PBMC infected with a recombinant vaccinia virus expressing HIV-1_{LAI}Env/Gag/Pol antigens and supplemented with 10U/ml rIL-2 (Hoffman-La Roche, Nutley, NJ) every 3-4 d. After 10-14 d, cells in each well were assayed for cytolytic activity against autologous target cells infected with wild type vaccinia or recombinant vaccinia expressing HIV-1_{LAI}Env antigens or HIV-1_{LAI}Gag/Pol. Wells were considered positive if cytolysis exceeded the mean spontaneous release from that target by 3 SD. CTL frequencies and 95% confidence intervals were determined by maximum likelihood analysis with software provided by S Kalams, Harvard Medical School [16]. Target cells were autologous B lymphoblastoid cell lines (BLCL), established from each macaque by infecting PBMC with *H. papio*, a baboon herpresvirus [9]. BLCL could not be transformed with PBMC of one control animal (M4) and could not be maintained in long term culture from one vaccinated animal (M10) and CTL data could not be generated from those animals.

EXAMPLE 6

Analysis of HIV-1 DNA and viral levels

HIV-1 *gag* and HLA-DQ DNA were amplified from extracted DNA form PBMC samples and quantified using primer pairs SK38/39 and GH26/27 (Gibco-BRL) respectively using PCR conditions as described [9]. DNA from 10^5 PBMC was standardised according to the DQ band density in comparison to 8E5 cell DNA (which contains 1 HIV-1 DNA copy/cell) and confirmed by measuring absorbance on a spectrophotometer (Ultrospec 3000, Pharmacia Biotech) at 260nm. Virus isolation was performed by cocultivating 10^6 macaque PBMC with 10^6 PHA-stimulated pooled human PBMCs and 50U/ml IL-2. Fresh media and IL-2 were added to the cultures twice weekly and PHA-stimulated human PBMC added weekly for 4 weeks. HIV-1 was quantified in cultures supernatants by HIV-1 p24 EIA (Abbott Laborators, Abbott Park, IL).

EXAMPLE 7

Safety of FPV expressing IFNγ

Locally delivered cytokines encoded by viral vectors are generally less toxic than systemically administered cytokines [8]. We analysed the reactogenicity of FPVgag/pol-IFN-γ in comparison to 4 matched controls not immunized and a control animal receiving FPVgag/pol-IFNγ only. A high dose of the FPV vaccines was administered (10^8 FPU) in an attempt to detect any significant adverse effects. A 44-75% reduction in activity of all 3 FPV-immunised macaques was observed for the first 24 hrs following vaccination, and in one of two FPVgag/pol-IFNγ immunised animal (M9), 28% reduction of activity was present between 24 to 48 hrs, but was normal thereafter in all animals. Swelling at the injection site was observed for 1-2 days following vaccinations in all 3 FPV vaccinated animals. No fever was documented following the FPV vaccinations (Figure 3a). All animals gained weight normally. No change in $CD4^+$ or $CD8^+$ T cell subsets, or monocyte levels in PBMC were observed following vaccination by (Figure 3b). Additionally, no significant changes in plasma electrolytes, renal function as assessed by plasma creatinine and urea, liver function markers, haemoglobin, white cell counts or

platelet counts were observed following FPVgag/pol or FPVgag/pol-IFNγ vaccination.

EXAMPLE 8

T cell immunogenicity

To determine whether vaccination with FPVgag/pol-IFNγ enhanced Gag/pol specific Th responses, macaques infected with HIV-1 9 months previously were vaccinated twice with FPVgag/pol-IFNγ (2 animals, M9 and M10) or FPVgag/pol (1 animal, M7). Th proliferative response to p24 Gag protein was enhanced 4-7 fold 1-2 weeks after the first FPVgag/pol-IFNγ vaccination and was greater than baseline levels 3 months later (Figure 4a). Following a second FPVgag/pol-IFNγ vaccination, p24-specific Th responses were further boosted above baseline (5-30 fold) and maintained for at least a further 2 months. The animal which received 2 FPVgag/pol immunisations had a 3 fold enhancement of p24-specific Th response. Tetanus-specific Th responses did not change following FPV vaccinations (<3 fold variation over time). The Th responses to Gag or tetanus antigens of 4 control macaques (M2, M3, M4, M5) did not change, with a <2 fold variation over the 4 month observation period (means SI to p24 was 3.2 and to Tetanus toxoid 3.6).

We also assessed whether FPVgag/pol-IFNγ vaccination of HIV-1 infected animals was associated with a change in the phenotype of Gag-specific Th response. Enhanced IFNγ secretion, but not IL-4 secretation, by Gag-specific Th responses from PBMC of animals receiving both FPVgag/pol and FPVgag/pol-IFNγ was observed, with the magnitude of the modulation of the cytokine secretion being greater in the FPVgag/pol-IFNγ immunised animals (Figure 4b). No change in the tetanus-specific Th phenotype from animals M7, M9 and M10 was observed following FPV vaccinations, with IL-4 secretion exceeding that of IFNγ (by 4-12 fold) both before and 2-6 weeks after FPV vaccinations of all 3 FPV vaccinated animals.

EXAMPLE 9

HIV-specific CTL activity following FPVgag/pol-IFNγ immunisation

Considerable interest currently focuses on immunisation strategies to maintain CTL responses in the face of marked reduction in antigenic stimulus from replicating HIV-1 [2, 5]. HIV-1 specific CTL response in macaques parallel the reduction in HIV-1 DNA following the first few months of HIV-1 infection, and in the "latent" phase HIV-1 specific CTL responses are low (≤10 HIV specific CTLs/10^6 PBMC) [9]. By a limiting dilution analysis, CTL precursors to Gag/Pol (but not Env) antigens were enhanced from <5 to $15/10^6$ PBMC following one FPVgag/pol-IFNγ vaccination and to $44/10^6$ PBMC following a second FPVgag/pol-IFNγ vaccination (Figure 3). Gag/Pol or Env specific CTLs were not detectably enhanced (remaining $\leq 5/10^6$ PBMC) in controls animals either unvaccinated (M2, M3, M5) or vaccinated with FPVgag/pol-IFNγ without IFNγ (M7, Figure 5).

EXAMPLE 10

HIV-1 levels following vaccination

To determine whether FPVgag/pol-IFNγ vaccination altered HIV-1 viral levels in macaques previously infected with HIV-1, HIV-1 DNA and culturable virus were studied before and after vaccinations. Using *env*-specific primers, animals M7, M9 and M10 had ≤10 copies of HIV-1 DNA/10^5 PBMC 0, 1 and 4 months prior to vaccinations and remained at ≤10 copies of HIV-1 DNA/10^5 PBMC at 1, 2 and 4 weeks following the first FPV vaccination, without detectable changes in HIV-1 DNA levels. HIV-1 could not be recovered from cocultured PBMC from any of the 3 vaccinated FPV animals either prior to (weeks 0, -4) or following (weeks +1, +2, +4, +6) vaccination. The cocultured method employed has routinely recovered HIV-1 when plasma HIV-1 RNA levels were 100-400 copies [(9); (10)], suggesting a significant rise in HIV-1 plasma RNA did not occur.

EXAMPLE 11

HIV-1 antibody levels

Gag/Pol specific antibodies were also enhanced following the 2 FPV vaccinations (Figure 6). p24-specific antibodies were enhanced in all 3 vaccinated animals, with no difference observed between the FPVgag/pol-IFNγ and FPVgag/pol vaccinated animals. No change in gp120 antibody responses was observed.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. A recombinant viral construct comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV antigen.

2. The recombinant viral construct according to claim 1 wherein said HIV is HIV-1.

3. The recombinant viral construct according to claim 2 wherein said HIV-1 antigen is Gag and/or Pol.

4. The recombinant viral construct according to claims 1, 2 or 3 wherein said cytokine is γ-interferon.

5. The recombinant viral construct according to claims 1, 2 or 3 wherein said cytokine is IL-2.

6. The recombinant viral construct according to any one of claims 1-5 wherein said avipox virus is fowl pox virus.

7. The recombinant viral construct according to any one of claims 1-5 wherein said avipox virus is canary pox virus.

8. The recombinant viral construct according to claim 1 wherein said avipox viral vector is fowl pox virus, said HIV-1 antigens are Gag and/or Pol and said cytokine is γ-interferon.

9. A vaccine composition comprising an avipox viral vector or functional derivative thereof which incorporates a first nucleic acid molecule encoding one or more HIV antigens or derivatives thereof and a second nucleic acid molecule encoding a cytokine or functional derivative thereof wherein said recombinant viral construct is effective in inducing, enhancing or otherwise stimulating an immune response to said HIV antigen.

10. The vaccine composition according to claim 9 wherein said HIV is HIV-1.

11. The vaccine composition according to claim 10 wherein said HIV-1 antigen is Gag and/or Pol.

12. The vaccine composition according according to claims 9, 10 or 11 wherein said cytokine is γ-interferon.

13. The vaccine composition according to claims 9, 10 or 11 wherein said cytokine is IL-2.

14. The vaccine composition according to any one of claims 9-13 wherein said avipox virus is fowl pox virus.

15. The vaccine composition according to any one of claims 9-13 wherein said avipox virus is canary pox virus.

16. The vaccine composition according to claim 9 wherein said avipox viral vector is fowl pox virus, said HIV-1 antigens are Gag and/or Pol and said cytokine is γ-interferon.

17. A pharmaceutical composition comprising the recombinant viral construct according to any one of claims 1-8 together with one or more pharmaceutically acceptable carriers and/or diluents.

18. A method of inducing, enhancing or otherwise stimulating, in a mammal, an immune response to HIV said method comprising administering to said mammal an effective amount of the viral construct according to any one of claims 1-8 for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

19. The method according to claim 18 wherein said HIV is HIV-1.

20. The method according to claim 19 wherein said viral construct is a viral construct according to claim 3.

21. A method of inducing, enhancing or otherwise stimulating, in a mammal, an immune response to HIV said method comprising administering to said mammal an effective amount of the vaccine composition according to any one of claims 9-16 for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

22. The method according to claim 21 wherein said HIV is HIV-1.

23. The method according to claim 22 wherein said vaccine composition is a composition according to claim 11.

24. A method of treating a mammal, said method comprising administering to said mammal an effective amount of the viral construct according to any one of claims 1-8 for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

25. The method according to claim 24 wherein said HIV is HIV-1.

26. The method according to claim 25 wherein said viral construct is a viral construct according to claim 3.

27. A method of treating a mammal, said method comprising administering to said mammal an effective amount of the vaccine composition according to any one of claims 1-8 for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

28. The method according to claim 27 wherein said HIV is HIV-1.

29. The method according to claim 28 wherein said viral construct is a viral construct according to claim 3.

30. A method for the treatment and/or prophylaxis of HIV infection or AIDS in a mammal said method comprising administering to said mammal an effective amount of the recombinant viral construct according to any one of claims 1-8 for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

31. The method according to claim 30 wherein said HIV is HIV-1.

32. The method according to claim 31 wherein said viral construct is a viral construct according to claim 3.

33. A method for the treatment and/or prophylaxis of HIV infection or AIDS in a mammal said method comprising administering to said mammal an effective amount of the vaccine composition according to any one of claims 9-16 for a time and under conditions sufficient to induce, enhance or otherwise stimulate an immune response to one or more HIV antigens.

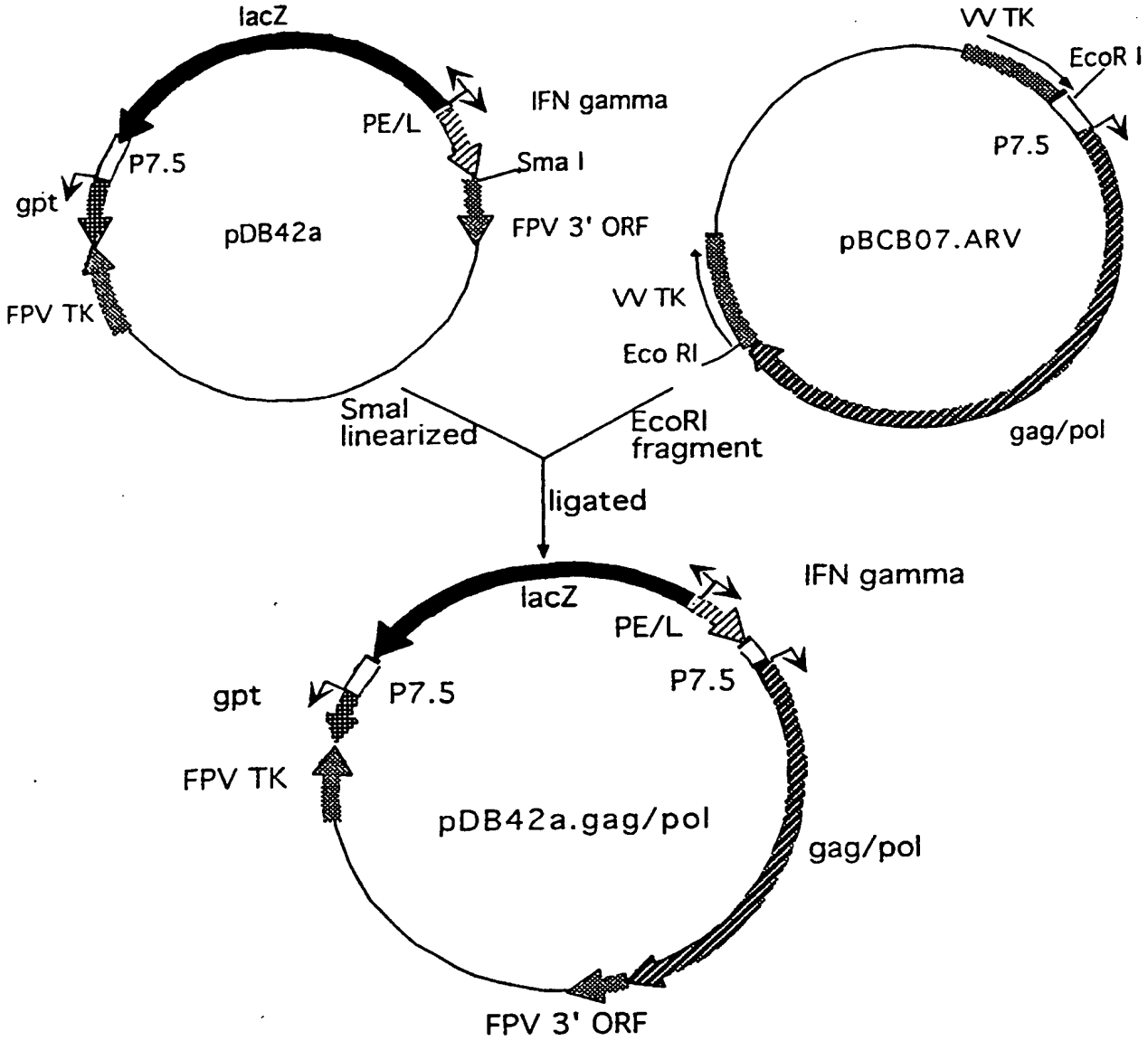
34. The method according to claim 33 wherein said HIV is HIV-1.

35. The method according to claim 34 wherein said vaccine composition is a composition according to claim 11.

36. Use of a recombinant viral construct according to any one of claims 1-8 in the manufacture of a medicament for the therapeutic and/or prophylactic treatment of HIV infection.

37. Use according to claim 36 wherein said HIV is HIV-1.

38. An agent useful for inducing, enhancing or otherwise stimulating, in a mammal, an immune response to HIV said agent comprising a recombinant viral construct according to any one of claims 1-8.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VV TK
Eco RI
gag/pol
SmaI
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Figure 1a

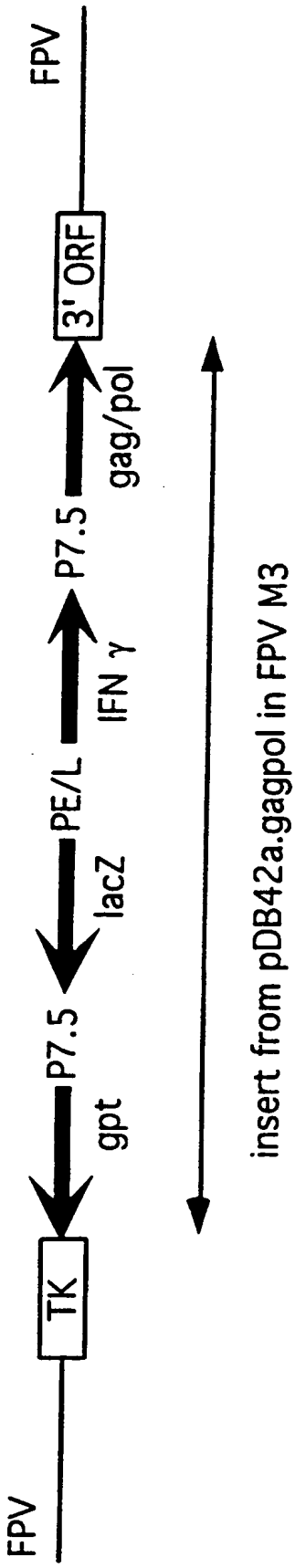
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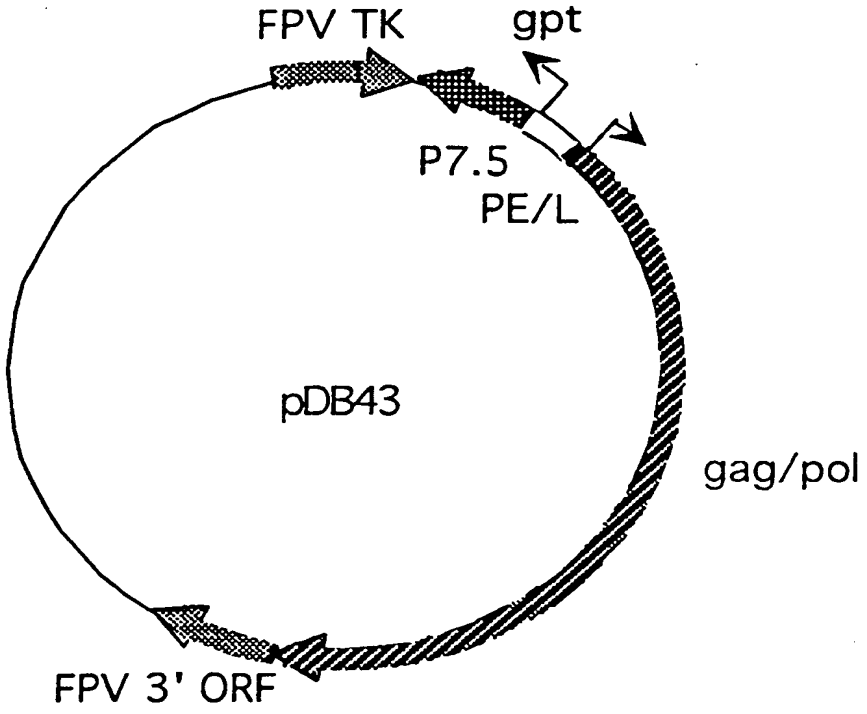
Figure 1b



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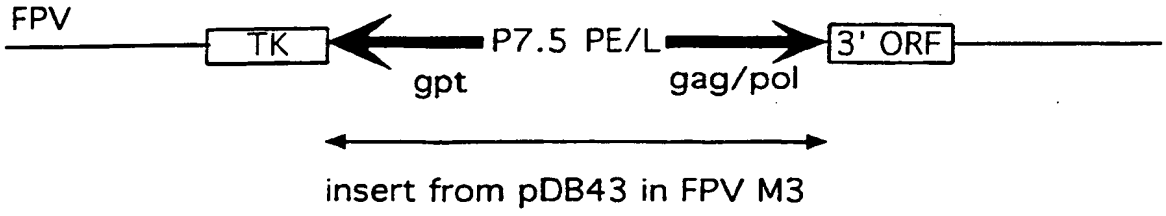
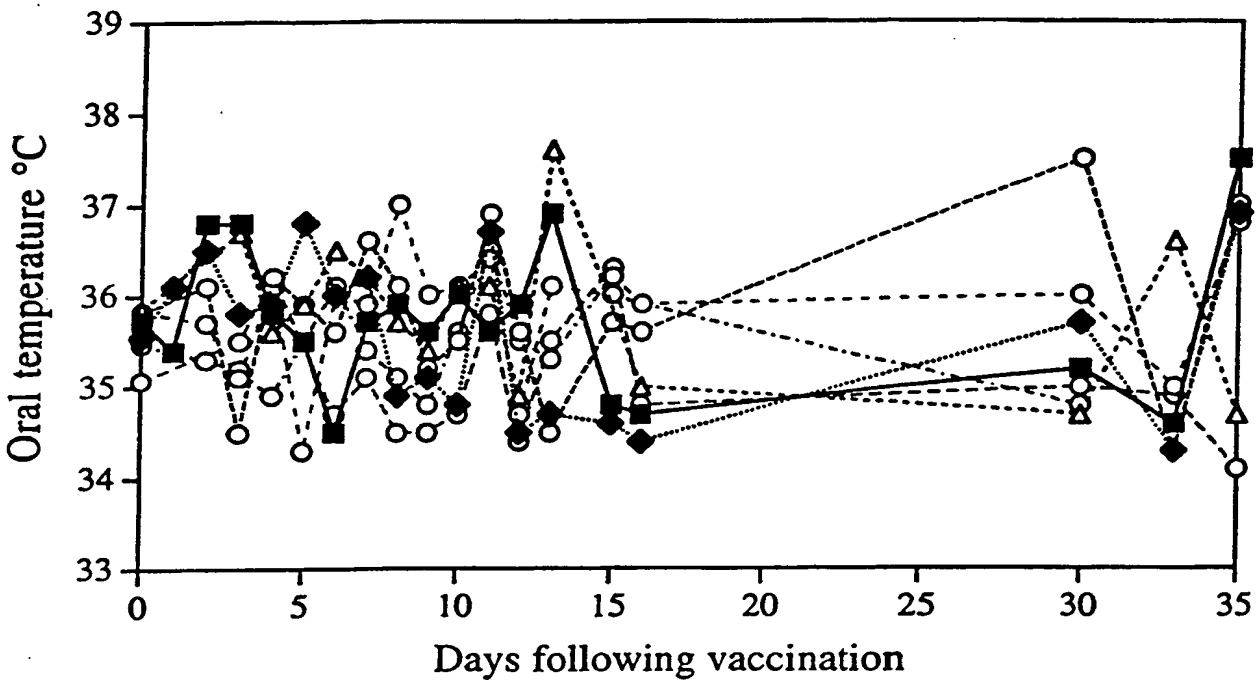


Figure 2b

Figure 3a



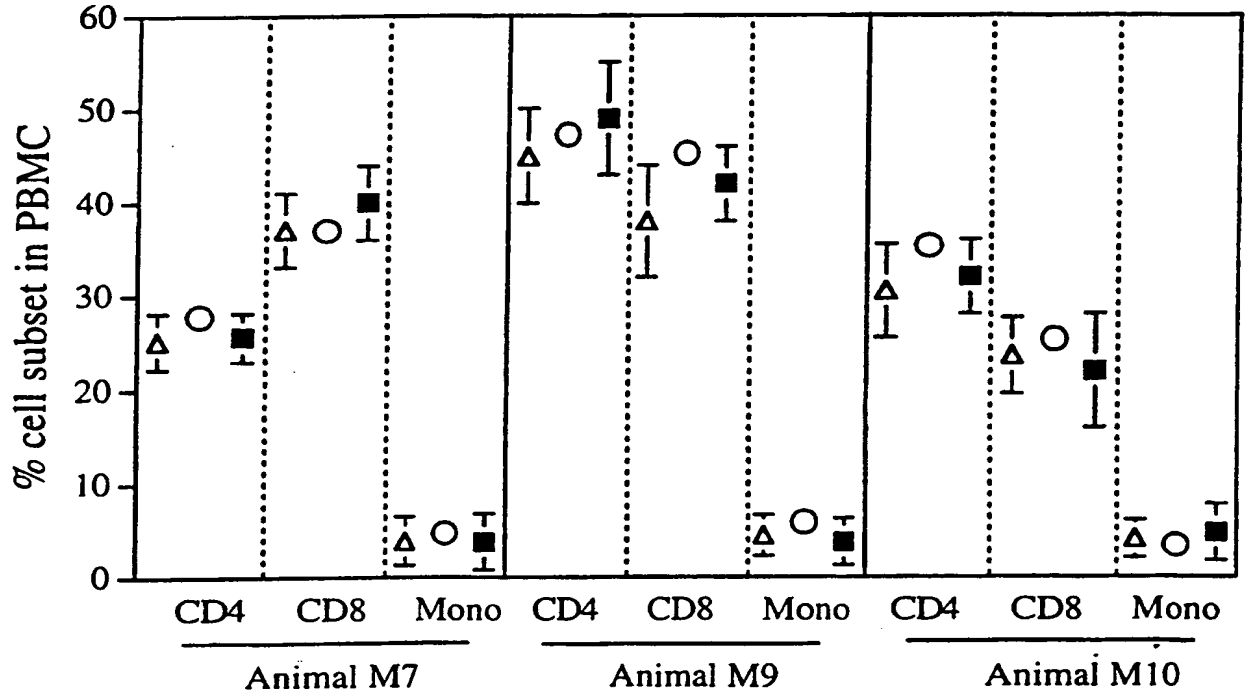
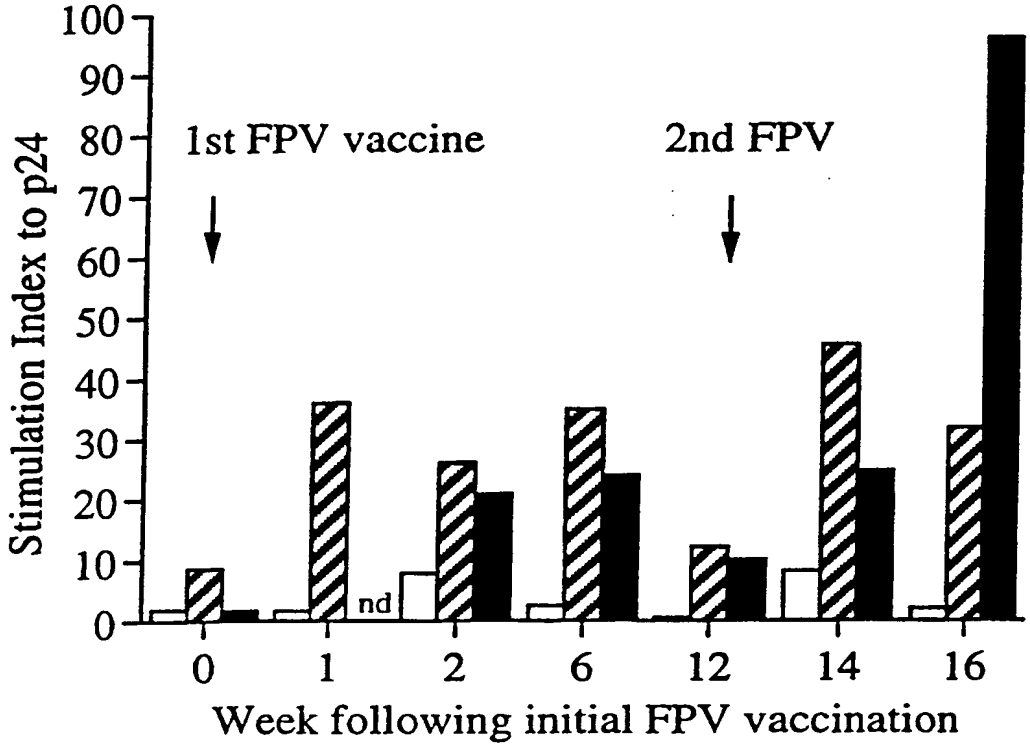


Figure 3b

Figure 4a



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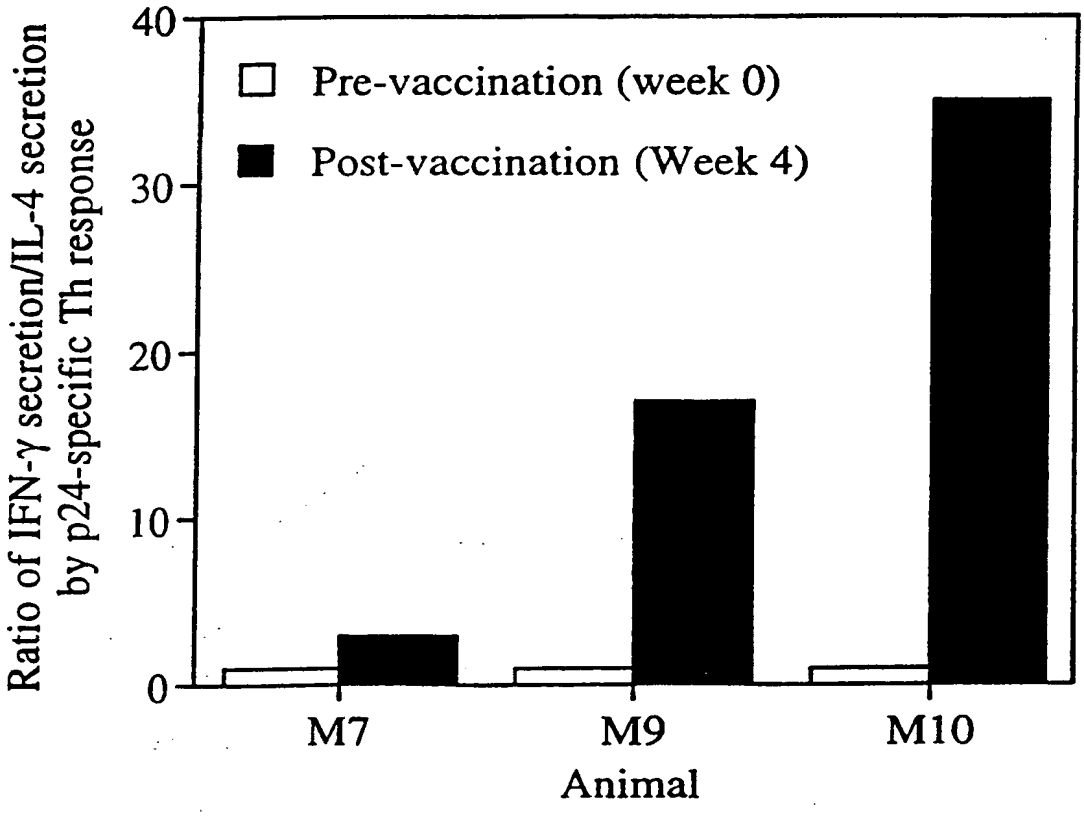

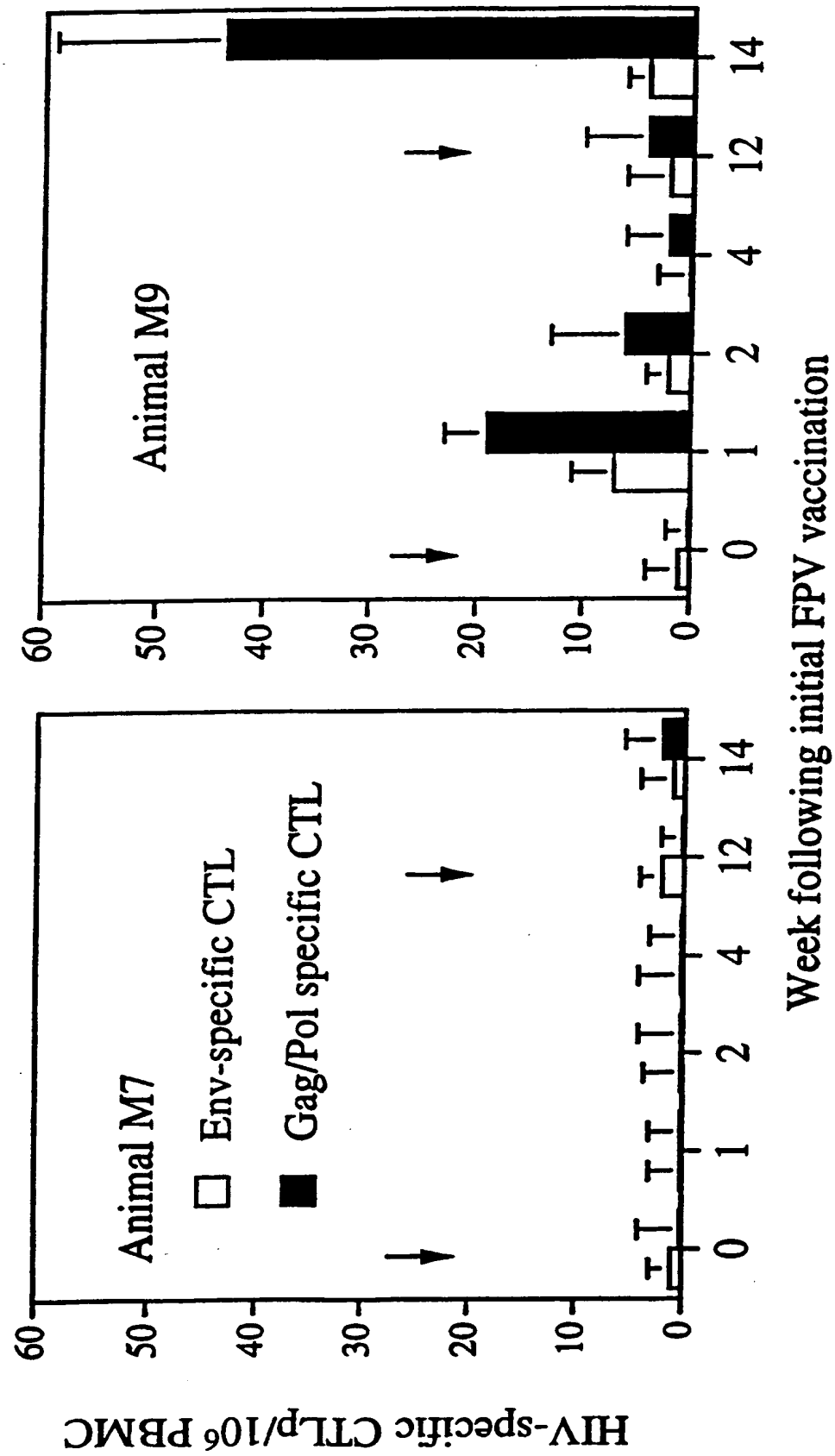
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Animal M7
Animal M9
Env-specific CTL
Gag/Pol specific CTL
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20
30
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60
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2
4
12
14

Figure 5

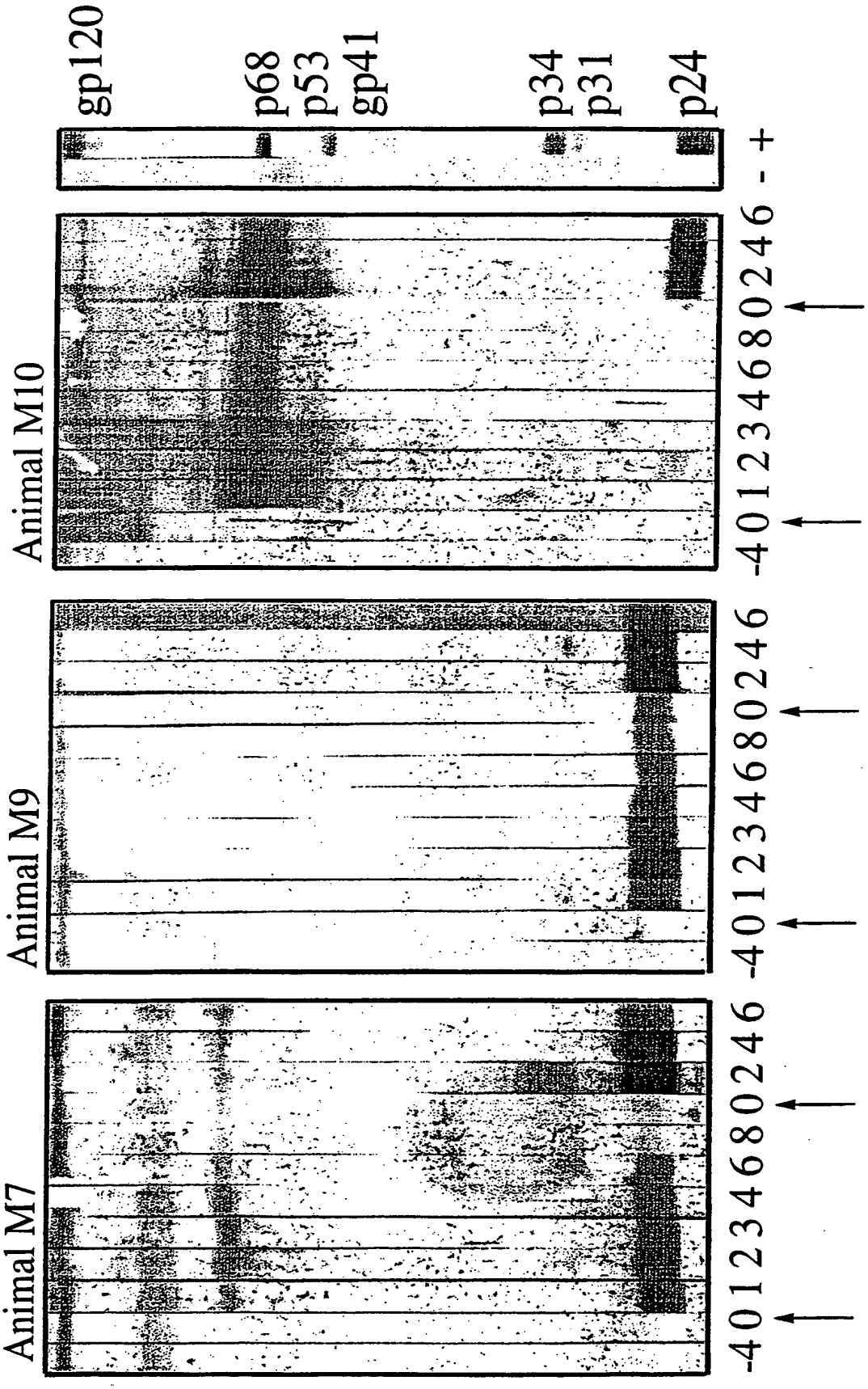
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Figure 6

SEQUENCE LISTING

<110> The Macfarlane Burnet Centre for Medical Research Limited;
Commonwealth Scientific and Industrial Research Organisation
The Australian National University

<120> Recombinant Viral Constructs and Methods Relating
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2231646	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 99/00989	International filing date *(day/month/year)* 9 November 1999	(Earliest) Priority Date *(day/month/year)* 9 November 1998

Applicant

The Macfarlane Burnet Centre for Medical Research Limited et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

Avipox vector coding an HIV antigen and a Cytokine

5. With regard to the **abstract,**

☐ the text is approved as submitted by the applicant

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention

☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00989

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The invention relates to a fowl pox virus encoding an HIV antigen (gag and/or pol) and a cytokine (γ-interferon).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00989

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 7/00 A61K 39/275

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE ELECTRONIC DATA BASE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: ((Pox()virus OR Poxvirus) AND Avian AND Vaccine) OR {(Vector* OR Recombinant()virus) AND (Pox()virus OR Poxvirus) AND (Avian OR HIV OR Cytokine OR Interferon: OR Interleukin:)}
Medline, CA: (Genetic Vectors/CT AND (Avipoxvirus/CT OR (HIV AND Cytokines/CT))

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/22641 (VIROGENETICS CORPORATION) 23 December 1992 Whole Document	1 - 38
X	WO 94/16716 (VIROGENETICS CORPORATION) 4 August 1994 Whole Document	1 - 38
Y	Ruby, J. et al, *Vaccine Research*, 1(4), (1992) pp347 - 356 "Recombinant Virux Vectors That Coexpress Cytokines - A New Vaccine Strategy" Whole Document	1 - 38

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 1999	20 DEC 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **J.H. CHAN** Telephone No.: (02) 6283 2340

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00989

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kim, J. J; et al The Journal of Immunology 158 (1997) pp 816 - 826 "In vivo Engineering of a Cellular Immune Response by Coadministration of IL-12 Expression Vector with a DNA Immunogen" Whole Document, Results p 817, & Discussion pp823-825	1 - 38

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00989

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	94/16716	AU	61652/94	US	5833975	US	5942235
		EP	680331				
WO	92/22641	AU	22597/92	US	5766598	US	5863542
		EP	592546				

END OF ANNEX